의학박사 학위논문

일시적으로 혈뇌장벽이 열린 래트 모델에서 Paclitaxel과 Rapamycin의 신경독성 연구

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이 논문을 의학과 박사학위논문으로 제출함

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ABSTRACT

Neurotoxicity of Paclitaxel and Rapamycin in Rat Model with Transient Blood-Brain Barrier Disruption

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Background: Drug-eluting stents and balloons are occasionally used for patients with medically intractable intracranial atherosclerotic stenosis to reduce postprocedural restenosis. However, neurotoxicity by the drugs are not clear yet.

Objective: The authors aim to find out whether drugs impregnated in stents and balloons cause neurotoxicity in a rat model with brain-blood barrier (BBB) disruption

Methods: A rat model was made with intra-arterial catheter indwelling at the right common carotid artery for drug administration. Optimal time interval was searched for transient BBB opening after mannitol administration. Paclitaxel and rapamycin in different doses equivalent to human doses of 600, 1200 and 2400 μg were administered via intra-arterial catheter in an optimal time interval from mannitol

injection, respectively. Brain tissues were obtained at 24 hours and 14 days after drug administration for a total of 60 rats, consisting of 6 groups per drug and 5 rats per group. Additionally, a total of 10 brain tissues were obtained from 2 sham groups of each 5 rats in 24 hours and 14 days after drug administration. All the rats were evaluated in terms of neurological status and histological findings for neuron damage and inflammation.

Results: Optimal time interval for BBB disruption was determined as 10 minutes after mannitol administration. Injecting each drugs in 10 minutes after mannitol administration via the intra-arterial catheter, there were no significant findings of neuronal damage and inflammation in all the cases, irrespective of the type of drugs, drug concentration and time interval between drug injection and histologic examination. None showed neurological deficits.

Conclusion: Intra-arterial injection of paclitaxel and rapamycin, ranging from the original to quadruple doses equivalent to human, did not cause neurotoxicity in rats with transient BBB disruption. This animal data is expected to be applicable in the human condition.

Keywords: Intracranial atherosclerotic stenosis, Neurotoxicity, Paclitaxel, Rapamycin, Restenosis

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Introduction

Intracranial atherosclerotic stenosis (ICAS) is one of major causes of the ischemic stroke.[1] According to the initial study for ICAS,[2,3] about 24% of the patients experienced an ischemic stroke during the follow-up with aspirin® administration. Meanwhile, initial studies with endovascular intervention showed a lower rate of ischemic stroke in about 7% of the patients treated with bare metal stents.[1,4] However, as similar with the initial percutaneous coronary intervention, about 20%-30% of the patients is known to have experienced an in-stent restenosis more than 50% in diameter in about 6 months after the intervention for ICAS. In order to overcome the in-stent and postprocedural restenosis, physicians have developed drug-eluting stents (DESs) and balloons (DEBs) which release drugs inhibiting intimal hyperplasia and they could achieve a significant reduction in the incidences of myocardial infarction and postprocedural restenosis. Subsequently, animal and clinical studies for ICAS also showed similar results of a low restenosis rate less than 5% using DESs for coronary heart diseases (CHD).[5-11]

Since the approval of Wingspan stent system® (Stryker, Fremont, CA, USA) by Food and Drug Administration in 2005,[12] 3 preliminary clinical studies showed a high technical success rate and a low rate of 30-day ischemic stroke in about 6%-9%.[13-15] However, the promising mood for intervention was discouraged with the opposite results in the randomized controlled study comparing best medical treatment and intervention,[16] in which 30-day and 1-year stroke rates were 14.7% versus 5.8%, and 20% versus 12.2% between best medical and stent groups, respectively. Nonetheless, recent studies showed satisfactory results using different kinds of stents and undersized balloon,[17-20] and another retrospective comparative

study with the Wingspan stent showed a similar stroke rate between medical and stent groups.[21] In addition, a randomized controlled prospective study is under way for the symptomatic ICAS more than 70%.[22] Most of all, as intra-arterial thrombectomy has become a first modality for acute stroke with major artery occlusion,[23] the safety and effectiveness are expected to improve much more with the aid of newly-designed endovascular devices such as stent and balloon, and well-designed clinical studies.

During the past 10-year use of DESs and DEBs for CHD, there has been no report about neurological problems. However, drugs directly released within the intracranial vessels are thought to be able to cause the neurotoxicity as a first-pass effect in each manner of slow but persistent release from DESs and bolus release from DEBs. Moreover, neurotoxicity can become profound in the stroke patients with disrupted blood-brain barrier (BBB). Clinical use of DESs and DEBs for ICAS has rarely but persistently reported. However, there are just few animal studies about the neurotoxicity related with DESs.[10,11] The authors aimed to find out the neurotoxicity by the drugs used for DESs and DEBs in a rat model with BBB disruption.

Materials and Methods

Animal Model

Male Sprague-Dawley specific pathogen-free rats were obtained from Bio-

Genomics (Seoul, South Korea). Rats were at 7-8 months of age and 250-350 g of body weight. The animals were housed in a conventional state under adequate temperature (23℃) and humidity (60%) control with a 12-hour light / 12-hour dark cycle for 1 week, and provided with free access to food and water. The procedures for handling and caring for animals adhered to the guidelines that are in compliance with the current international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985, revised 1996), and they were approved by the Institutional Animal Care and Use Committee of Kangwon National University. All of the experiments were conducted to minimize the number of animals used and the suffering caused by the procedures used in the present study.

Rats were anesthetized with an intraperitoneal injection of 30 mg/kg of Zoletil® (Virbac, Carros, France). Subcutaneously, 4 mg/kg of gentamicin was injected before skin incision to prevent from infection and 3 mg/kg of ketoprofen was administered after procedures to relieve the pain. Perioperatively, routine check-up of body weight, diet, behavior and neurological status was performed daily for 4 days after procedures and every 2 days after procedures. When abrupt loss of body weight more than 20% of the preoperative weight or abnormal behavior are observed, the rat was to be euthanized.

After the shaving and sterilization with betadine solution of the cervical skin area, right common, external and internal carotid arteries were exposed. Right external carotid artery was tied with a thread to permanently occlude, and proximal and distal parts of right common carotid artery were temporarily clamped with clips. Puncturing the temporarily occluded vessel segment with a 24-gauge cannula,

intra-arterial infusion catheter was indwelled at the right common carotid artery. Head of the catheter was plugged up with a heparin cap and fixed at the surrounding soft tissues. Then, catheter was irrigated with heparinized saline to remove the air and blood clots via the heparin cap, and temporary clips were finally removed. When intra-arterial drug injection via the intra-arterial catheter was completed, the catheter was removed and puncture site was sutured with 10-0 nylon for bleeding control (**Figure 1**).

Temporary BBB Disruption

In order to make a similar condition of the chronically ischemic brain with partially disrupted BBB, temporary BBB disruption was attempted with intra-arterial infusion of mannitol. Rat model was designed to find out the optimal time interval between mannitol administration and drug infusion in which temporary BBB disruption was maximized and drug was able to pass through the BBB into the brain parenchyme.

A 20% of mannitol (CJ Health Care, Chungcheongbuk-do, Korea) was administered at a rate of 0.25 cc/kg/sec for 30 seconds with an infusion pump via the intra-arterial catheter. Subsequently in a few predetermined time intervals of 0, 5, 10, 15 and 20 minutes after mannitol administration, a 2% solution of Evans Blue (EB; Sigma, St Louis, MO, USA) in normal saline (4 mL/kg of body weight) was injected via the intra-arterial catheter. In 24 hours after EB infusion, a rat were perfused transcardially with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.4).[24] Then,

whole brain was extracted and the grade of extravasation of EB was evaluated seeing the right hemisphere. A total of 25 rats (5 cases per predetermined interval) were used. The grades of EB extravasation was scored as follows: grade 0, no EB extravasation; grade 1, pale; grade 2, moderate; and grade 3, strong (**Figure 2**).

Drug Administration

A main stock of 1 ml of 5 mg/ml paclitaxel (Sigma, St Louis, MO, USA) was made, mixing 5 mg of paclitaxel powder with 1 ml of 25 mg/ml dimethyl sulfoxide (Sigma, St Louis, MO, USA). Then, sub-stocks of 2 ml of 0.05 μg/μl paclitaxel were finally made, mixing the 2 μl of paclitaxel main stock with 2 ml of normal saline. Similarly, a main stock of 200 μl of 5 mg/ml rapamycin (Sigma, St Louis, MO, USA) was made, dissolving 1mg of rapamycin powder with 200 μl of 25 mg/ml dimethyl sulfoxide. Then, sub-stocks of 2 ml of 0.05 μg/μl rapamycin were finally prepared, mixing 20 μl of rapamycin main stock with 2 ml of normal saline.

Dosage of drugs such as paclitaxel and rapamycin was translated from human into animal equivalent doses using a formula based on body surface area: animal equivalent dose (mg/kg) = human dose (mg/kg) x human K_m / animal K_m.[25] The 60 kg-weighted human and 250 g-weighted rat K_m are 37 and 6, respectively. For example, 250 g-weighted rat equivalent doses of human doses of 600, 1200 and 2400 μg in a 60 kg-weighted human are 15.4, 30.8 and 61.7, respectively. As a dose of 600 μg is maximal contained in the surface of a commercial DEB, comparative dosage were determined.[26] Each rat equivalent dosage was calculated and injected, using the formula with a body weight of each rat.

Drugs of paclitaxel and rapamycin were injected together with EB via the intra-arterial catheter in an optimal time interval after mannitol administration, respectively. Calculated animal doses of drugs equivalent to human doses of 600, 1200 and 2400 μg, were infused in an intra-arterial bolus manner, and then brain tissues were obtained in each 24 hours and 14 days after drug infusion. A total of 70 rats was needed, consisting of 6 groups per drug, 5 rats per group and 2 drugs of paclitaxel and rapamycin, including 2 sham groups (5 rats per group) for each 24 hours and 14 days after drug-free solution infusion after mannitol administration.

Neurological and Histologic Examination

Neurological status was evaluated using Bederson et al.'s grading system as follows: grade 0, no observable deficit; grade 1, forelimb flexion; grade 2, decreased resistance to lateral push without circling; and grade 3, same behavior as grade 2, with circling.[27]

For the histological examination, rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and perfused trans-cardially with 0.1 M PBS (pH 7.4) followed by 4% paraformaldehyde in 0.1 M phosphate-buffer (pH 7.4). The brains were removed and post-fixed in the same fixative for 6 hours. The brain tissues were cryoprotected by infiltration with 30% sucrose overnight. Thereafter, frozen tissues were serially sectioned on a cryostat (Leica, Wetzlar, Germany) into 30-μm coronal sections, and they were then collected into six-well plates containing PBS.

For the confirmation of neuronal death, cresyl violet staining (Sigma, St Louis, MO, USA) was performed. The sections were mounted on gelatin-coated

microscope slides. Cresyl violet acetate (Sigma, St Louis, MO, USA) was dissolved at 1.0% (w/v) in distilled water, and glacial acetic acid was added to this solution. Before and after staining for 2 minutes at a room temperature, the sections were washed twice in distilled water. After dehydration, the sections were mounted with Canada balsam (Kanto, Tokyo, Japan).

Fluoro-Jade C (FJ C; Histochem, Jefferson, AR, USA) histofluorescence staining was conducted to localize the neuronal degeneration.[28] In brief, the sections were first immersed in a solution containing 1% sodium hydroxide in 80% alcohol, and followed in 70% alcohol. They were then transferred to a solution of 0.06% potassium permanganate, and transferred to a 0.0004% FJ C staining solution. After washing, the sections were placed on a slide warmer (approximately 50℃), and then examined using an epifluorescent microscope (Carl Zeiss, Oberkochen, Germany) with blue (450-490 nm) excitation light and a barrier filter. With this method neurons that undergo degeneration brightly fluoresce in comparison to the background.[29]

To obtain the accurate data for immunohistochemistry, the sections at 24 hours and 14 days after drug infusion were processed by immunohistochemistry under the same conditions. The sections were sequentially treated with 0.3% hydrogen peroxide in PBS for 30 minutes and 10% normal goat serum in 0.01 M PBS for 30 minutes. They were next incubated with diluted rabbit anti-glial fibrillay acidic protein (GFAP) (diluted 1:1,000; Biogenesis, San Ramon, CA, USA) for astrocytes and rabbit anti-ionized calcium-binding adapter molecule 1 (Iba-1) (diluted 1:500; Wako, Osaka, Japan) for microglia overnight at 4℃ and subsequently exposed to biotinylated goat anti-rabbit IgG and streptavidin peroxidase complex (diluted

1:200; Vector, Burlingame, CA, USA). They were visualized by staining with 3, 3'-diaminobenzidine in 0.1 M Tris-HCl buffer (pH 7.2) and mounted on gelatin-coated slides. After dehydration the sections were mounted in Canada balsam (Kanto, Tokyo, Japan).

The grades of neuronal damage were scored with the findings on cresyl violet and FJ C histofluorescence staining as follows: grade 0, no neuronal damage; grade 1, mild damage; grade 2, moderate damage; and grade 3, severe damage. The grades of inflammation were scored with the findings on GFAP and Iba-1 immunohistochemistry as same as those of neural damage.

Results

Optimal Time Interval for Transient BBB Disruption

The results of EB extravasation are summarized in **Table 1**. EB extravasation was identified from 10-minute to 20-minute intervals after bolus administration of mannitol via the carotid artery. The grades of EB extravasation were similar during that time periods. So, optimal time for drug injection in which BBB would be temporarily disrupted was determined as 10 minutes after mannitol administration.

Neurotoxicity of Paclitaxel and Rapamycin

Brain specimens were acquired in 1 day and 14 days after drug administration,

and striatum and hippocampus in the sliced tissues were observed. Most of rat brains were stained with EB, with a various range of grades from 1 to 3 (**Table 2**).

There were no significant histological abnormalities on the sham groups (**Table 3, Figure 3** and **4**). There were no significant findings of neuronal death and inflammation on both hemispheres of each experimental rat brains, irrespective of the type and concentration of drugs, and time intervals between drug administration and histologic examinations (**Figure 5-16)**. Merely on 1 or 2 of all the sectioned slices in each rat brain, focal neuronal damage and inflammation were observed, which is considered insignificant: moderate focal change on 1 slice sectioned in the No. 4 rat brain on the 14^{th} day after 600 μg human dose of paclitaxel; and mild focal change on 1 or 2 sectioned slices of the other affected hemispheres (**Table 5** and **6**, **Figure 17** and **18**). While taking care of rats after drug administration, none showed neurological abnormality in terms of the Bederson's criteria.

Discussion

Summary

When performing balloon angioplasty and stenting for the medically intractable ICAS, DESs or DEBs are sometimes used in order to reduce in-stent or postprocedural restenosis after using conventional devices as for CHDs. Long-term exposure of the brain to low dose of drugs which are slowly released from DEBs and DESs looks safe because there is no report about neurotoxicity in the clinical

setting for almost a decade. However, some patients with chronic ICAS can have partially disrupted BBB and first-pass effect by drugs released directly from the devices placed at the intracranial vessels can occur. Moreover, a high dose of drugs is known to be released into the bloodstream during the first angioplasty with DEBs. So, the authors planned to carry out the experiment about the neurotoxicity by a bolus injection of high dose of drugs such as paclitaxel and rapamycin frequently used in DESs and DEBs in a rat model with BBB disruption. Drugs were administered in the original, doubled and quadruple doses equivalent to the maximal dose for human. Except for focal histological changes of neuronal damage and inflammation only on 1 or 2 of all the slices in each 11 rat brains, there was no significant abnormality on histologic examination. Additionally, there was no neurological deficit in any case. To the best of our knowledge, this study is the first animal experiment about the neurotoxicity by a dose of drugs impregnated in the DESs and DEBs. Although the role of neurointervention was reduced in patients with ICAS after the SAMMPRIS trial,[16] this data is considered to be an important reference in using DESs or DEBs for the unstable ICAS.

Historical perspectives in development of devices for CHD and their applications for ICAS

The history of coronary intervention, as mentioned above, includes balloon angioplasty alone, balloon angioplasty and stenting for reducing post-ballooning acute occlusion and restenosis, and DESs and DEBs for reducing in-stent and postprocedural restenosis.[30-32] As the first generation of DESs, TAXUS® (Boston Scientific co., Natick, MA, USA) and CYPHER® (Cordis co., Warren, NJ, USA)

were introduced, respectively impregnated with paclitaxel and rapamycin. Thereafter, new generation stents with low profile struts, new derivatives of rapamycin and absorbable materials are subsequently being introduced. Currently, about less than 15% of in-stent restenosis and 2% of delayed in-stent restenosis is known about the DESs.[33-35] Similar with the DESs, DEBs have also shown satisfactory results, with some merits over DESs such as no occurrence of delayed in-stent thrombosis and stent fracture at the peripheral vessels, no need of long-term use of dual antiplatelets, and application at the thin vessels and bifurcation areas where it is difficult to deliver stents.[35-38]

DESs have been infrequently applied in patients with ICAS and they showed promising results. According to the Gupta et al.'s study, the rate of in-stent restenosis more than 50% in diameter was about 5% in 4 months, using TAXUS® and CYPHER® for symptomatic ICAS with a mean stenosis of 83%.[5] The other studies also reported low rates of in-stent restenosis, ranging from 0% to 0.5%.[6-9] However, there were some concerns about delayed in-stent thrombosis and neurotoxicity by the drugs. Most of all, SAMMPRIS trial showed a superiority of best medical treatment to stent insertion.[16] Some probable drawbacks were discussed: operators' inexperience, problems of Wingspan stent®, high severity of included patients with severe stenosis and early treatment within 30 days of events, and rigorous adjustment process. Nonetheless, expectations about the neurointervention was abruptly regressed and medical treatment was established as a standard treatment of choice. Because the authors were planning this study at that period, they were seriously worried whether to conduct this experiment or not.

However, there still seem chances to turn the previous results of neurointervention

for the ICAS. Recent retrospective studies showed satisfactory results using other kinds of stents and undersized balloon angioplasty.[17,18] A study with a large number of patients using Wingspan stent® reported similar results between stent and medical groups.[21] A prospective study in China is on-going, applying to the patients with symptomatic ICAS more than 70% stenosis and stroke recurrence within 90 days of best medical treatment.[22] In addition, newly designed stents or other devices are expected to result in better outcomes. Recently, angioplasty alone with no stent placement for the symptomatic ICAS showed favorable outcomes.[19,20] DEBs can be a good alternative to stents with distally accessible intermediate catheter through which early release of drugs can be minimized.

Mechanisms of drugs and characteristic of DESs and DEBs

According to some researches about the mechanism of restenosis after stent insertion and balloon angioplasty,[39,40] aggregation of fibrin and platelets occurs within the initial 2 days after procedure, inflammatory cells such as neutrophil and macrophage gather between 2 and 10 days, migration and proliferation of smooth muscle cells occurs within 2 and 14 days, endothelization is completed in 10 to 14 days, and vessels are finally stabilized over 2 weeks after extracellular matrix is formed. Here, smooth muscle cells are the main cause of restenosis and they are the target of drugs impregnated in stents and balloons.

Typical drugs for DESs and DEBs are paclitaxel known as anticancer drug and rapamycin as immunosuppressant after organ transplantation. Paclitaxel inhibits cell division in G2/M phase, then it stabilizes polymerized microtubules and

minimize neointimal hyperplasia by inhibiting replication of smooth muscle cells.[41] Also, paclitaxel is lipophilic and highly attachable at the vessel walls that it was used for first generation DESs and DEBs.[42,43] Rapamycin mainly has a cytostatic effect of arresting late G1 phase by combining the membrane protein kinase named as mammalian target of rapamycin. Additionally, it is known for inhibiting inflammation, cell migration and extracellular matrix.[44]

TAXUS® stents are composed of stent strut and polymer coated with paclitaxel of 1 μg /mm^2 (a total of 85-155 μg).[44] Half of paclitaxel is released within the initial 48 hours after stent placement and it is slowly released over 2 and 3 months. CYPHER® stents are coated with a total of 71-315 μg rapamycin and they are known to have a wide therapeutic range because there was no toxic effect upto 1200 μg of rapamycin in an animal study.[44] On the other hand, currently produced DEBs are containing paclitaxel and typical commercial one is SeQuent Please (B. Braun, Melsungen, Germany). It is coated with 3 μg/mm^2 (a total of 300-600 μg) of paclitaxel. On balloon inflation, about 75%-80% of paclitaxel is released into the bloodstream, 10% is retained in the balloon and the other 10%-15% is attached to the targeted vessel wall, generating the protective effect against neointimal hyperplasia over 2 weeks.[26] In this study, the original human dose of 600 μg was determined based on the maximal dose on DEBs.

Paclitaxel-related complications are known to increase in proportion to drug concentration and various kinds of side effects begin to occur when drug concentration exceeds 250 mg/m^2. Most of complicated cases related to the nervous system are peripheral neuropathy and just 2 cases related to the central nervous system are reported.[45] Additionally, paclitaxel is known not to pass through

the normal BBB in animal and human studies.[45,46] Meanwhile, rapamycin goes well through the BBB and its neurotoxicity is reported only in an *in vitro* experiment.[47,48] There are no neurotoxicity reported in *in vivo* studies with humans and animals.[10,11,49] Rather, an attention has been paid to rapamycin's protective effects on neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, aging, neuroinflammation and neuronal death.[50-52] DESs and DEBs have been used for more than 10 years and there has been no report about the neurological complications. In this study with a rat model, there was no evidence of neurotoxicity related to a high dose of drugs. This data is expected to be helpful in clinical implication of DESs and DEBs for symptomatic ICAS.

Limitations

First, just prototype of drugs for DESs and DEBs were used. New generation devices are impregnated with newer derivatives with more selective and safer properties. As most of newer drugs are derivatives of rapamycin and paclitaxel is still used in DEBs, this data would be applicable in using new generation devices for ICAS. Second, an *in vitro* experiment was not conducted with human cells of neuron and glial cells. However, *in vitro* experiments are just a former step for an *in vivo* study and drug concentrations in *in vitro* experiments are not easy to be properly adjusted in an *in vivo* study. Third, although neurointervention became limited in ICAS since the results of SAMMPRIS trial, technical advancement is expected to overcome the weakness of the present devices and this data would be subsequently more useful in the near future. Fourth, the degree of BBB disruption in rat models was not coherent, which means different drug passage via the

disrupted BBB. However, there was no tendency of neurotoxicity related with degree of BBB disruption as well as types of drug, drug concentration and time periods between drug administration and tissue acquisition. So, the authors did not perform additional experiments to meet the degree of BBB disruption, sacrificing more animals.

Conclusion

The authors could not encounter the histologic and neurological evidences of neurotoxicity in most of rats with BBB disruption after direct injection of paclitaxel and rapamycin via the intra-arterially indwelled catheter. There was no influencing factors such as type of drug (rapamycin and paclitaxel), drug concentration (original, doubled and quadruple doses equivalent to human) and time interval between drug injection and acquisition of brain tissue (1 and 14 days). The role of neurointervention has become a little limited for the patients with ICAS since the SAMMPRIS trial was presented. For the time being, this data may be limitedly useful in treating the patients with medically intractable ICAS with DES and DEB. However, this data is expected to be more helpful for the safety of drugs to reduce in-stent and postprocedural restenosis, with the advent of newly designed devices and techniques in the near future.

References

1. SSYLVIA Study Investigators. Stenting of Symptomatic Atherosclerotic Lesions in the Vertebral or Intracranial Arteries (SSYLVIA): study results. *Stroke.* 2004;35:1388-1392.

2. Chimowitz MI, Kokkinos J, Strong J, Brown MB, Levine SR, Silliman S, et al. The Warfarin-Aspirin Symptomatic Intracranial Disease Study. *Neurology.* 1995;45:1488–1493.

3. The Warfarin-Aspirin Symptomatic Intracranial Disease (WASID) Study Group. Prognosis of patients with symptomatic vertebral or basilar artery stenosis. *Stroke.* 1998;29:1389–1392.

4. Miao ZR, Feng L, Li S, Zhu F, Ji X, Jiao L, et al. Treatment of symptomatic middle cerebral artery stenosis with balloon-mounted stents: long-term follow-up at a single center. *Neurosurgery.* 2009;64:79-84.

5. Gupta R, Al-Ali F, Thomas AJ, Horowitz MB, Barrow T, Vora NA, et al. Safety, feasibility, and short-term follow-up of drug-eluting stent placement in the intracranial and extracranial circulation. *Stroke.* 2006;37:2562-2566.

6. Abou-Chebl A, Bashir Q, Yadav JS. Drug-eluting stents for the treatment of intracranial atherosclerosis: initial experience and midterm angiographic follow-up. *Stroke.* 2005;36:e165–e168.

7. Qureshi AI, Kirmani JF, Hussein HM, Harris-Lane P, Divani AA, Suri MF, et al. Early and intermediate-term outcomes with drug-eluting stents in high-risk patients with symptomatic intracranial stenosis. *Neurosurgery.* 2006;59:1044-1051.

8. Steinfort B, Ng PP, Faulder K, Harrington T, Grinnell V, Sorby W, et al. Midterm outcomes of paclitaxel-eluting stents for the treatment of intracranial posterior circulation stenoses. *J Neurosurg.* 2007;106:222-225.

9. Park S, Lee DG, Chung WJ, Lee DH, Suh DC. Long-term Outcomes of Drug-eluting Stents in Symptomatic Intracranial Stenosis. *Neurointervention.* 2013;8:9-14.

10. Levy EI, Hanel RA, Howington JU, Nemes B, Boulos AS, Tio FO, et al. Sirolimus-eluting stents in the canine cerebral vasculature: a prospective, randomized, blinded assessment of safety and vessel response. *J Neurosurg.* 2004;100:688-694.

11. Levy EI, Hanel RA, Tio FO, Garlick DS, Bailey L, Cunningham MR, et al. Safety and pharmacokinetics of sirolimus-eluting stents in the canine cerebral vasculature: 180 day assessment. *Neurosurgery.* 2006;59:925-933.

12. Food and Drug Administration. Wingspan stent system and gateway PTA balloon catheter: summary of safety and probable benefit. http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/MedicalDevices/MedicalDevicesAdvisoryCommittee/NeurologicalDevicesPanel/UCM296521.pdf. Accessed January 19th, 2016.

13. Fiorella D, Levy EI, Turk AS, Albuquerque FC, Niemann DB, Aagaard-Kienitz B, et al. US multicenter experience with the wingspan stent system for the treatment of intracranial atheromatous disease: periprocedural results. *Stroke.* 2007;38:881-887.

14. Bose A, Hartmann M, Henkes H, Liu HM, Teng MM, Szikora I, et al. A novel,

self-expanding, nitinol stent in medically refractory intracranial atherosclerotic stenoses: the Wingspan study. *Stroke.* 2007;38:1531–1537.

15. Zaidat OO, Klucznik R, Alexander MJ, Chaloupka J, Lutsep H, Barnwell S, et al. The NIH registry on use of the Wingspan stent for symptomatic 70–99% intracranial arterial stenosis. *Neurology.* 2008;70:1518–1524.

16. Chimowitz MI, Lynn MJ, Derdeyn CP, Turan TN, Fiorella D, Lane BF, et al. Stenting versus aggressive medical therapy for intracranial arterial stenosis. *N Engl J Med.* 2011;365:993-1003.

17. Lee KY, Chen DY, Hsu HL, Chen CJ, Tseng YC. Undersized angioplasty and stenting of symptomatic intracranial tight stenosis with Enterprise: Evaluation of clinical and vascular outcome. *Interv Neuroradiol.* 2016;22:187-195.

18. Feng Z, Duan G, Zhang P, Chen L, Xu Y, Hong B, et al. Enterprise stent for the treatment of symptomatic intracranial atherosclerotic stenosis: an initial experience of 44 patients. *BMC Neurol.* 2015;15:187.

19. Connors JJ 3rd, Wojak JC. Percutaneous transluminal angioplasty for intracranial atherosclerotic lesions: evolution of technique and short-term results. *J Neurosurg.* 1999;91:415–423.

20. Marks MP, Marcellus ML, Do HM, Schraedley-Desmond PK, Steinberg GK, Tong DC, et al. Intracranial angioplasty without stenting for symptomatic atherosclerotic stenosis: long-term follow-up. *Am J Neuroradiol.* 2005;26:525–530.

21. Li TX, Gao BL, Cai DY, Wang ZL, Zhu LF, Xue JY, et al. Wingspan Stenting for Severe Symptomatic Intracranial Atherosclerotic Stenosis in 433 Patients

Treated at a Single Medical Center. *PLoS One.* 2015;10:e0139377.

22. Wang Y, Miao Z, Wang Y, Zhao X, Gao P, Liu L, et al. Protocol for a prospective, multicentre registry study of stenting for symptomatic intracranial artery stenosis in China. *BMJ Open.* 2014;4:e005175.

23. Goyal M, Menon BK, van Zwam WH, Dippel DW, Mitchell PJ, Demchuk AM, et al. Endovascular thrombectomy after large-vessel ischaemic stroke: a meta-analysis of individual patient data from five randomised trials. *Lancet.* 2016;387:1723-1731.

24. Kaya M, Gulturk S, Elmas I, Kalayci R, Arican N, Kocyildiz ZC, et al. The effects of magnesium sulfate on blood-brain barrier disruption caused by intracarotid injection of hyperosmolar mannitol in rats. *Life Sci.* 2004;76:201-212.

25. Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. *FASEB J.* 2008;22:659-661.

26. Scheller B1, Speck U, Abramjuk C, Bernhardt U, Böhm M, Nickenig G. Paclitaxel balloon coating, a novel method for prevention and therapy of restenosis. *Circulation.* 2004;110:810-814.

27. Bederson JB, Pitts LH, Tsuji M, Nishimura MC, Davis RL, Bartkowski H. Rat middle cerebral artery occlusion: evaluation of the model and development of a neurologic examination. *Stroke.* 1986;17:472-476.

28. Candelario-Jalil E, Alvarez D, Merino N, Leon OS. Delayed treatment with nimesulide reduces measures of oxidative stress following global ischemic brain injury in gerbils. *Neurosci Res.* 2003;47:245-253.

29. Schmued LC, Hopkins KJ. Fluoro-Jade B: a high affinity fluorescent marker for the localization of neuronal degeneration. *Brain Res.* 2000;874:123-130.

30. Ellis SG, Myler RK, King SB III, Douglas JS Jr, Topol EJ, Shaw RE, et al. Causes and correlates of death after unsupported coronary angioplasty: implications for use of angioplasty and advanced support techniques in high-risk settings. *Am J Cardiol.* 1991;68:1447–1451.

31. Nobuyoshi M, Kimura T, Nosaka H, Mioka S, Ueno K, Yokoi H, et al. Restenosis after successful percutaneous transluminal coronary angioplasty: serial angiographic follow-up of 229 patients. *J Am Coll Cardiol.* 1988;12:616-623.

32. Fischman DL, Leon MB, Baim DS, Schatz RA, Savage MP, Penn I, et al. A randomized comparison of coronary stent placement and balloon angioplasty in the treatment of coronary artery disease. *N Engl J Med.* 1994;331:496-501.

33. Morice MC, Colombo A, Meier B, Serruys P, Tamburino C, Guagliumi G, et al. Sirolimus- vs paclitaxeleluting stents in de novo coronary artery lesions: the REALITY trial: a randomized controlled trial. *JAMA.* 2006;295:895-904.

34. Stone GW, Midei M, Newman W, Sanz M, Hermiller JB, Williams J, et al. Comparison of an everolimus-eluting stent and a paclitaxel-eluting stent in patients with coronary artery disease: a randomized trial. *JAMA.* 2008;299:1903-1913.

35. Lee JM, Park J, Kang J, Jeon KH, Jung JH, Lee SE, et al. Comparison among drug-eluting balloon, drug-eluting stent, and plain balloon angioplasty for the treatment of in-stent restenosis: a network meta-analysis of 11 randomized, controlled trials. *JACC Cardiovasc Interv.* 2015;8:382-394.

36. Unverdorben M, Kleber FX, Heuer H, Figulla HR, Vallbracht C, Leschke M, et al. Treatment of small coronary arteries with a paclitaxel-coated balloon catheter in the PEPCAD I study: are lesions clinically stable from 12 to 36 months? *EuroIntervention.* 2013;9:620-628.

37. Unverdorben M, Vallbracht C, Cremers B, Heuer H, Hengstenberg C, Maikowski C, et al. Paclitaxel-coated balloon catheter versus paclitaxel-coated stent for the treatment of coronary in-stent restenosis: the three-year results of the PEPCAD II ISR study. *EuroIntervention.* 2015;11:926-934.

38. Fanggiday JC, Stella PR, Guyomi SH, Doevendans PA. Safety and efficacy of drug-eluting balloons in percutaneous treatment of bifurcation lesions: the DEBIUT (drug-eluting balloon in bifurcation Utrecht) registry. *Catheter Cardiovasc Interv.* 2008;71:629–635.

39. Farb A, Sangiorgi G, Carter AJ, Walley VM, Edwards WD, Schwartz RS, et al. Pathology of acute and chronic coronary stenting in humans. *Circulation.* 1999;99:44-52.

40. Ferns GA, Avades TY. The mechanisms of coronary restenosis: insights from experimental models. *Int J Exp Pathol.* 2000;81:63-88.

41. Finn AV, Nakazawa G, Joner M, Kolodgie FD, Mont EK, Gold HK, et al. Vascular responses to drug eluting stents: importance of delayed healing. *Arterioscler Thromb Vasc Biol.* 2007;27:1500–1510.

42. Mori T, Kinoshita Y, Watanabe A, Yamaguchi T, Hosokawa K, Honjo H. Retention of paclitaxel in cancer cells for 1 week in vivo and in vitro. *Cancer Chemother Pharmacol.* 2006;58:665–672.

43. Axel DI, Kunert W, Goggelmann C, Oberhoff M, Herdeg C, Küttner A, et al. Paclitaxel inhibits arterial smooth muscle cell proliferation and migration in vitro and in vivo using local drug delivery. *Circulation.* 1997;96:636–645.

44. Costa MA, Simon DI. Molecular basis of restenosis and drug-eluting stents. *Circulation.* 2005;111:2257-2273.

45. Rowinsky EK, Eisenhauer EA, Chaudhry V, Arbuck SG, Donehower RC. Clinical toxicities encountered with paclitaxel (Taxol). *Semin Oncol.* 1993;20:1-15.

46. Lesser GJ, Grossman SA, Eller S, Rowinsky EK. The distribution of systemically administered [3H]-paclitaxel in rats: a quantitative autoradiographic study. *Cancer Chemother Pharmacol.* 1995;37:173-178.

47. Tyler B, Wadsworth S, Recinos V, Mehta V, Vellimana A, Li K, et al. Local delivery of rapamycin: a toxicity and efficacy study in an experimental malignant glioma model in rats. *Neuro Oncol.* 2011;13:700-709.

48. Serkova N1, Christians U, Flögel U, Pfeuffer J, Leibfritz D. Assessment of the mechanism of astrocyte swelling induced by the macrolide immunosuppressant sirolimus using multinuclear nuclear magnetic resonance spectroscopy. *Chem Res Toxicol.* 1997;10:1359-1363.

49. Maramattom BV, Wijdicks EF. Sirolimus may not cause neurotoxicity in kidney and liver transplant recipients. *Neurology.* 2004;63:1958-1959.

50. Bové J, Martínez-Vicente M, Vila M. Fighting neurodegeneration with rapamycin: mechanistic insights. *Nat Rev Neurosci.* 2011;12:437-452.

51. Li CY, Li X, Liu SF, Qu WS, Wang W, Tian DS. Inhibition of mTOR pathway

restrains astrocyte proliferation, migration and production of inflammatory mediators after oxygen-glucose deprivation and reoxygenation. *Neurochem Int.* 2015;83-84:9-18.

52. Srivastava IN, Shperdheja J, Baybis M, Ferguson T, Crino PB. mTOR pathway inhibition prevents neuroinflammation and neuronal death in a mouse model of cerebral palsy. *Neurobiol Dis.* 2016;85:144-154.

Table 1. Optimal Time Period of Blood-Brain Barrier Disruption after Mannitol Infusion.

Rat No.	Evans Blue injection time after mannitol administration (min)				
	0	5	10	15	20
1	0	0	1	1	0
2	0	0	2	2	2
3	2	0	1	1	3
4	0	0	3	2	3
5	0	3	2	1	3

Values are grades of brain staining by Evans Blue. The grades of Evans Blue extravasation was scored as follows: grade 0, no extravasation; grade 1, pale; grade 2, moderate; and grade 3, strong.

Table 2. Extravasation of Evans Blue in Rat Brains.

Day	Rat No.	Paclitaxel dose for human (μg)			Rapamycin dose for human (μg)			Control
		600	1200	2400	600	1200	2400	
1	1	3	3	3	1	0	2	1
	2	3	2	3	0	2	2	1
	3	3	3	3	1	3	0	2
	4	3	1	3	1	0	2	2
	5	3	2	3	1	3	2	0
14	1	1	0	3	0	0	3	2
	2	1	2	3	0	2	3	2
	3	2	3	1	0	2	3	3
	4	2	0	2	2	1	1	1
	5	3	2	2	0	2	1	3

Values are grades of brain staining by Evans Blue. The grades of Evans Blue extravasation was scored as follows: grade 0, no extravasation; grade 1, pale; grade 2, moderate; and grade 3, strong.

Table 3. Histologic Findings of Brain in Sham Groups of Rats.*

Day	Rat No.	Striatum	Hippocampus
1	1	0, 0	0, 0
	2	0, 0	0, 0
	3	0, 0	0, 0
	4	0, 0	0, 0
	5	0, 0	0, 0
14	1	0, 0	0, 0
	2	0, 0	0, 0
	3	0, 0	0, 0
	4	2, 2	2, 2
	5	0, 0	0, 0

Values are grades of neuronal damage and inflammation, respectively. The grades of neuronal damage and inflammation were scored as follows: grade 0, no neuronal damage or inflammation; grade 1, mild damage or inflammation; grade 2, moderate damage or inflammation; and grade 3, severe damage or inflammation.

*All the positive histologic findings were observed only on 1 or 2 of 15 slices of each right hemisphere.

Table 4. Histologic Findings of Brain by Intra-arterial Paclitaxel Injection in Rats.*

Day	Rat No.	Drug dose for human (μg)					
		600		1200		2400	
		Striatum	Hippocampus	Striatum	Hippocampus	Striatum	Hippocampus
1	1	0, 0	0, 0	0, 0	1, 0	0, 0	0, 0
	2	0, 0	0, 0	0, 0	1, 0	0, 0	0, 0
	3	0, 0	0, 0	0, 0	1, 0	0, 0	0, 0
	4	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	5	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
14	1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	3	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	4	2, 2	2, 2	1, 0	0, 0	0, 0	0, 0
	5	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0

Values are grades of neuronal damage and inflammation, respectively. The grades of neuronal damage and inflammation were scored as follows: grade 0, no neuronal damage or inflammation; grade 1, mild damage or inflammation; grade 2, moderate damage or inflammation; and grade 3, severe damage or inflammation.

*All the positive histologic findings were observed only on 1 or 2 of 15 slices of each right hemisphere.

Table 5. Histologic Findings of Brain by Intra-arterial Rapamycin Injection in Rats.*

Day	Rat No.	Drug dose for human (μg)					
		600		1200		2400	
		Striatum	Hippocampus	Striatum	Hippocampus	Striatum	Hippocampus
1	1	0, 0	0, 0	1, 0	1, 0	0, 0	0, 0
	2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	3	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	4	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	5	0, 0	0, 0	0, 0	0, 0	1, 0	1, 0
14	1	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	2	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	3	0, 0	0, 0	0, 0	0, 0	0, 0	0, 0
	4	0, 1	0, 0	1, 0	0, 0	0, 1	0, 1
	5	0, 0	0, 0	0, 0	0, 0	0, 1	0, 1

Values are grades of neuronal damage and inflammation, respectively. The grades of neuronal damage and inflammation were scored as follows: grade 0, no neuronal damage or inflammation; grade 1, mild damage or inflammation; grade 2, moderate damage or inflammation; and grade 3, severe damage or inflammation.

[*]All the positive histologic findings were observed only on 1 or 2 of 15 slices of each right hemisphere.

Figure 1. A rat model for drug infusion via the right internal carotid artery with intra-arterial catheter indwelling.

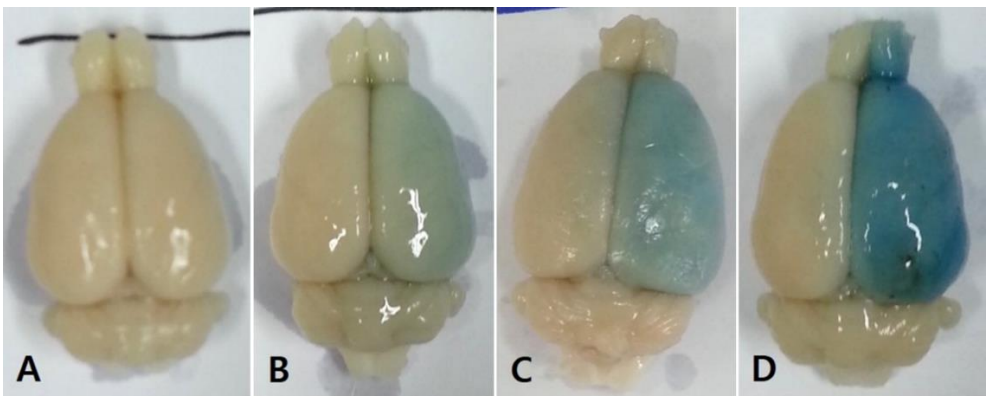

Figure 2. Grades of extravasation of Evans Blue in the right hemispheres of rat brains. A, grade 0 when there is no evidence of extravasation. B, grade 1 when the degree of extravasation is pale. C, grade 2 when the degree is moderate. D, grade 3 when the degree is strong.

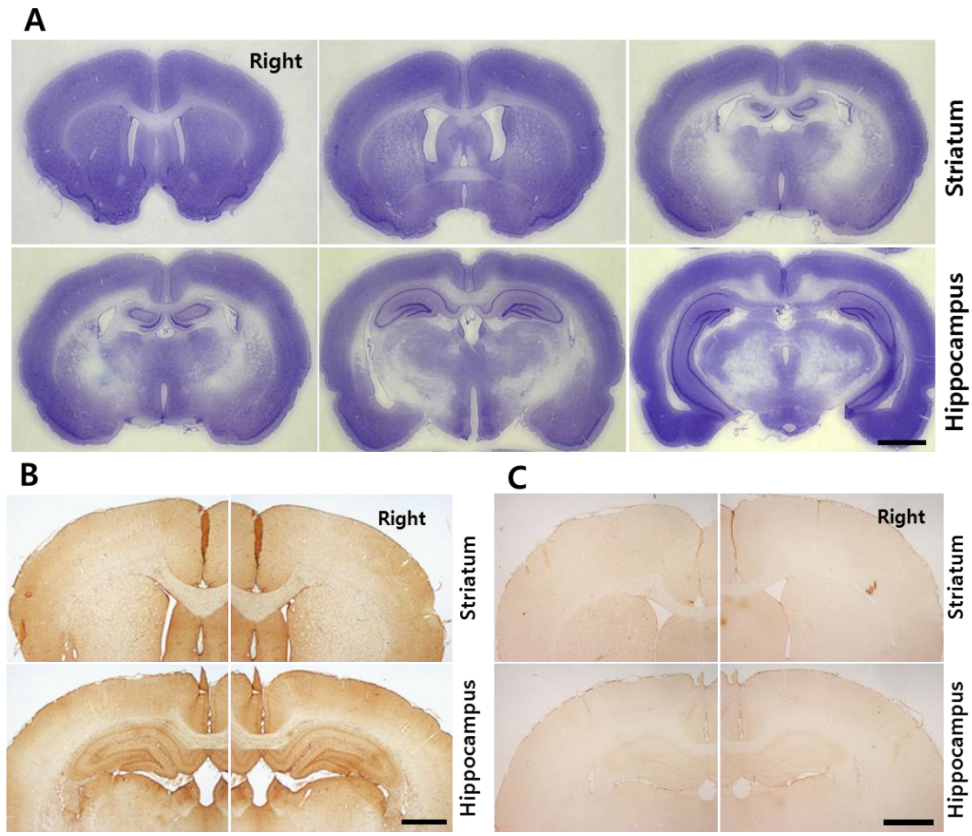

Figure 3. Cresyl violet (A), GFAP (B) and Iba-1 (C) staining of No. 1 rat brain of sham group on the 1st day. There are no evidences of brain damage. Scale bar = 2 mm.

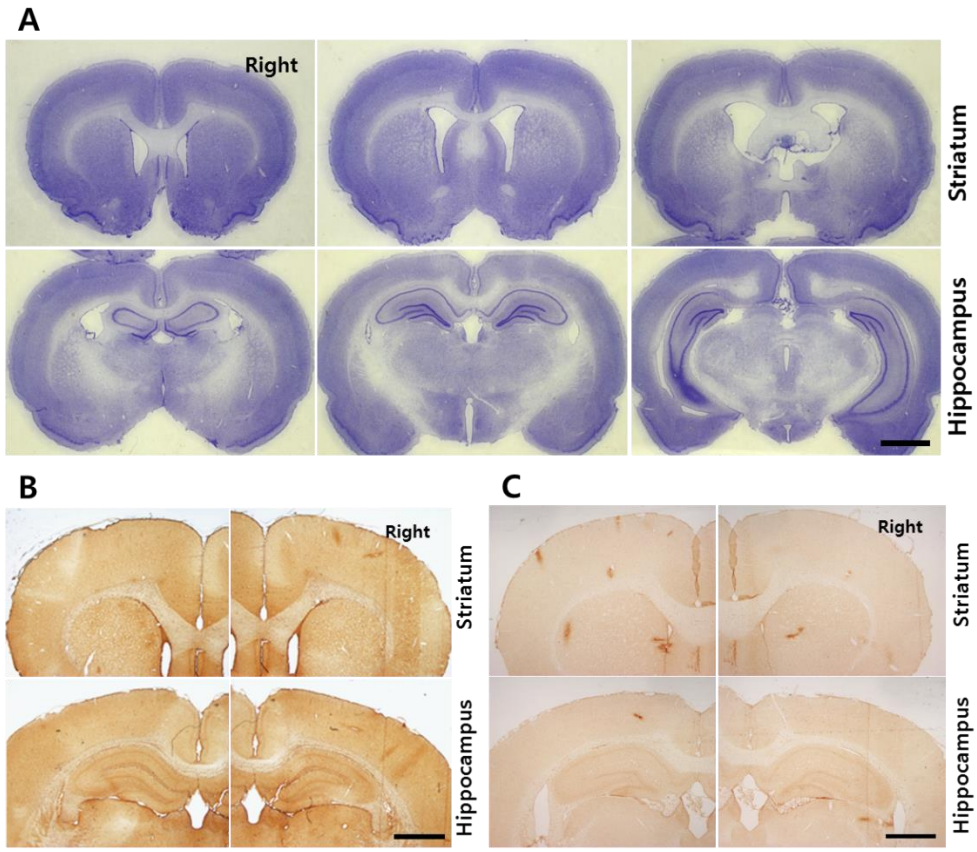

Figure 4. Cresyl violet (A), GFAP (B) and Iba-1 (C) staining of No. 1 rat brain of sham group on the 14th day. There are no evidences of brain damage. Scale bar = 2 mm.

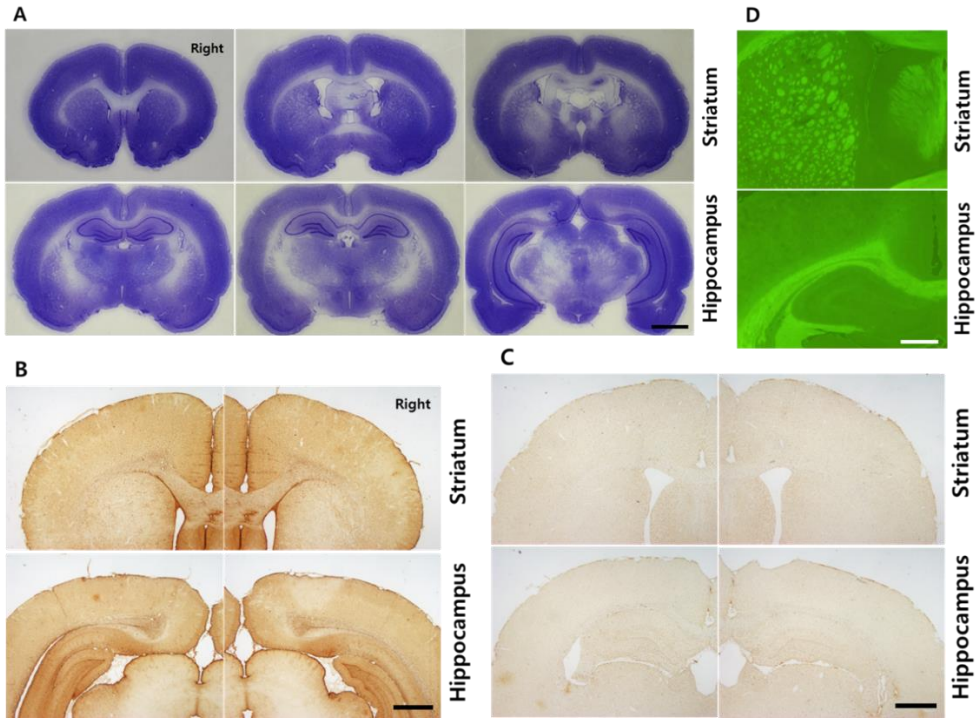

Figure 5. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 600 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

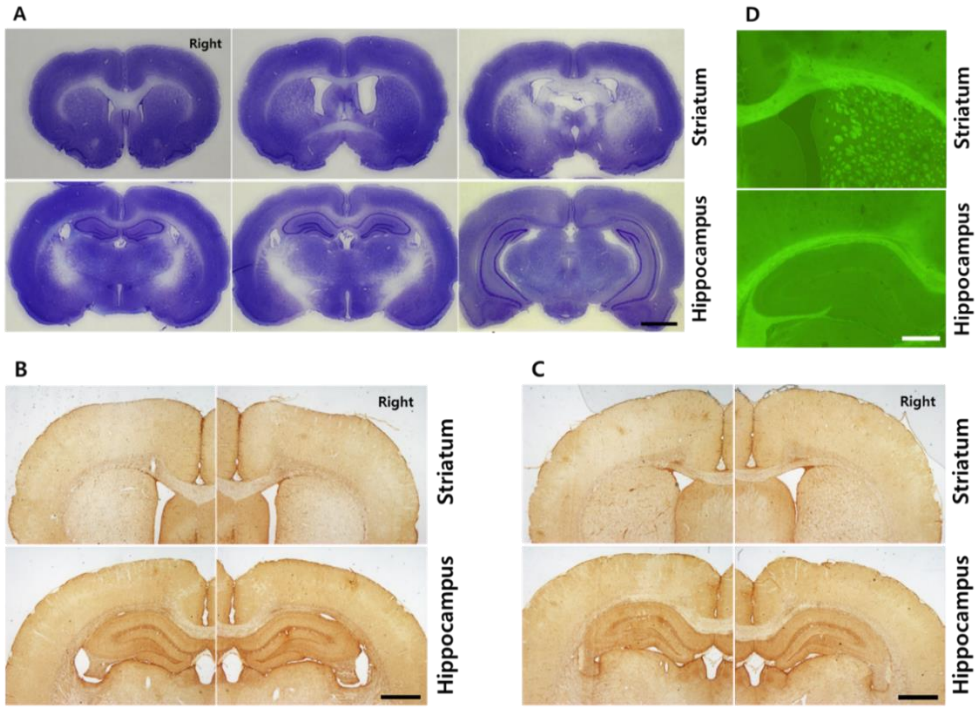

Figure 6. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 600 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

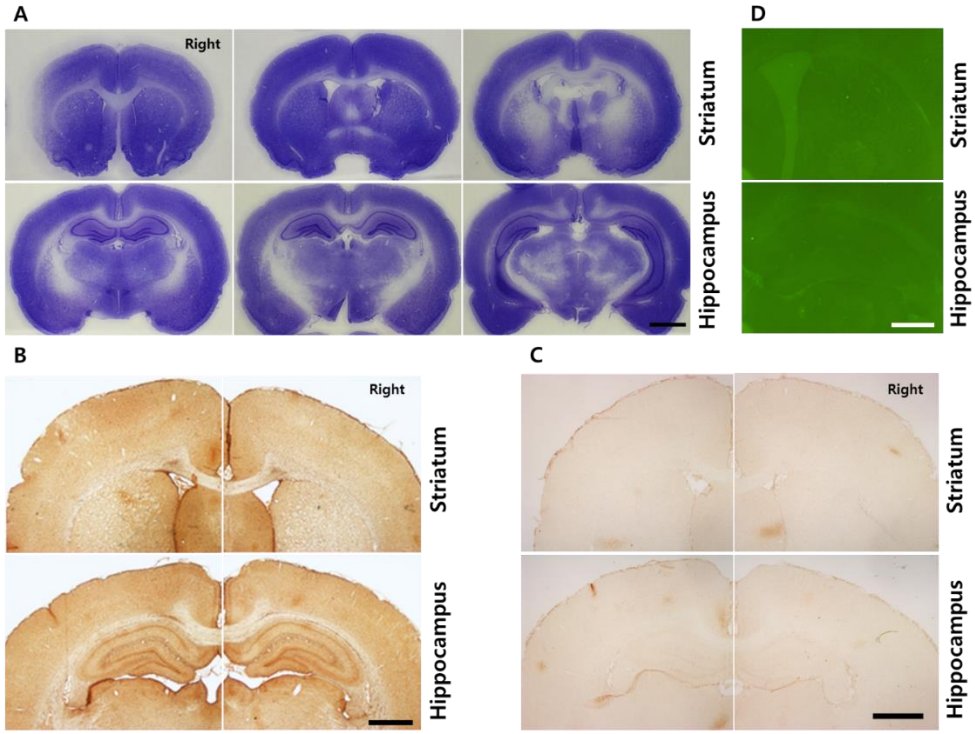

Figure 7. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 4 rat brain on the 1st day after 1200 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

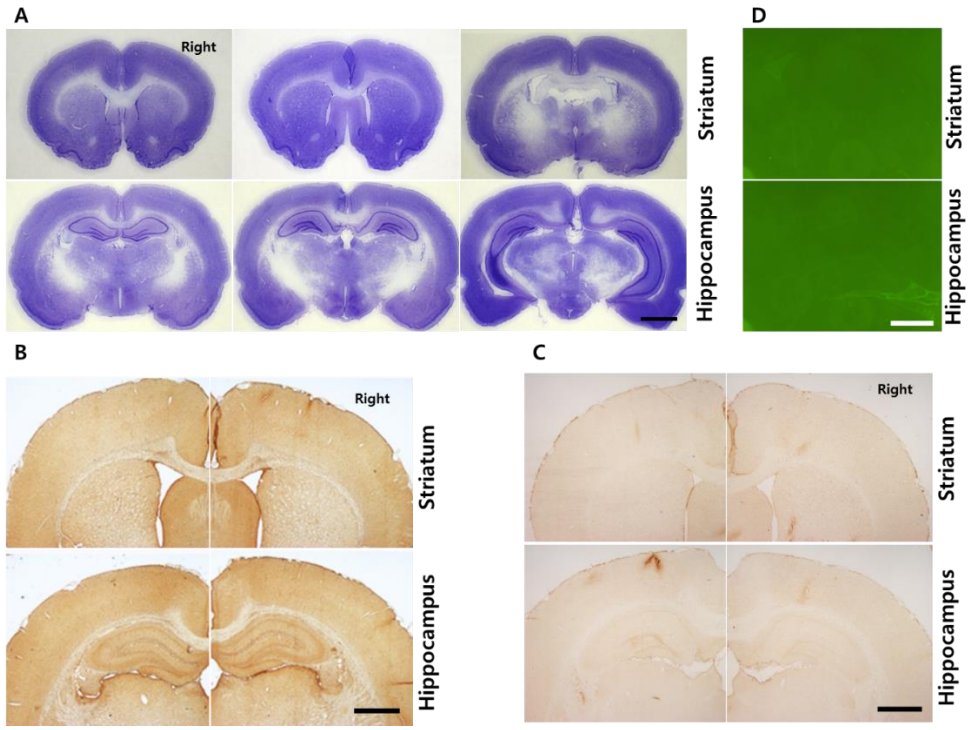

Figure 8. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 1200 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

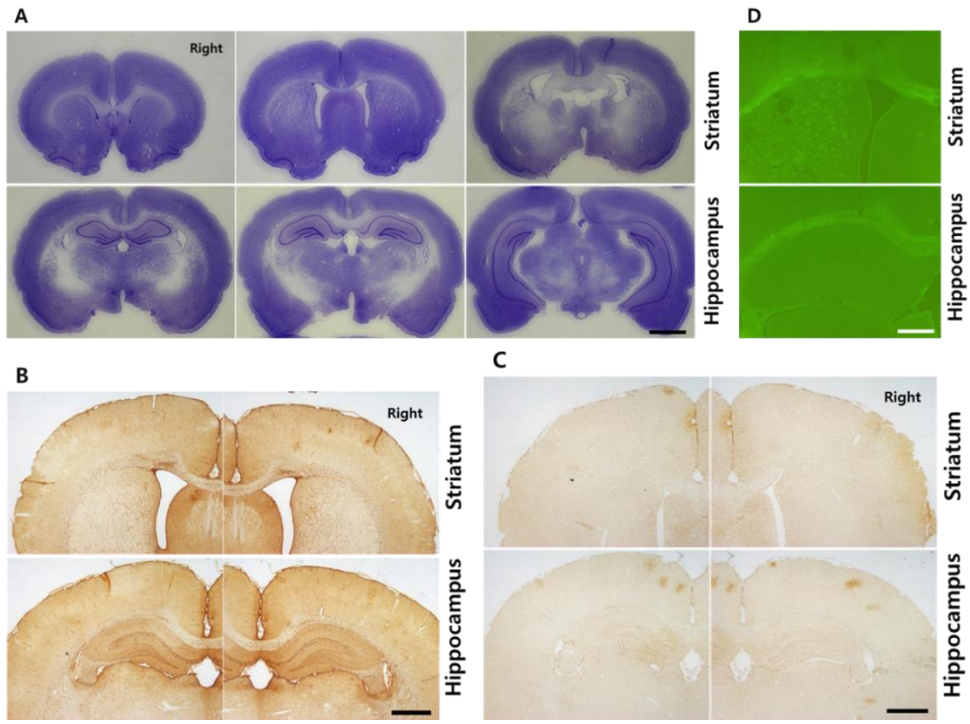

Figure 9. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 2400 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

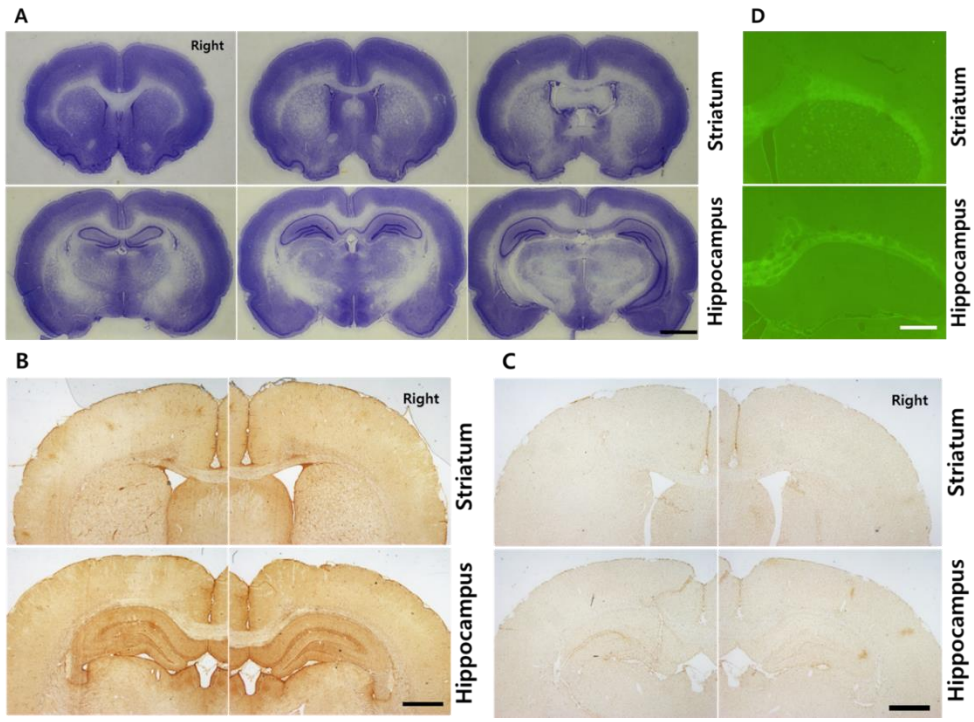

Figure 10. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 2400 μg human dose of Paclitaxel. There are no evidences of brain damage. Scale bar = 2 mm.

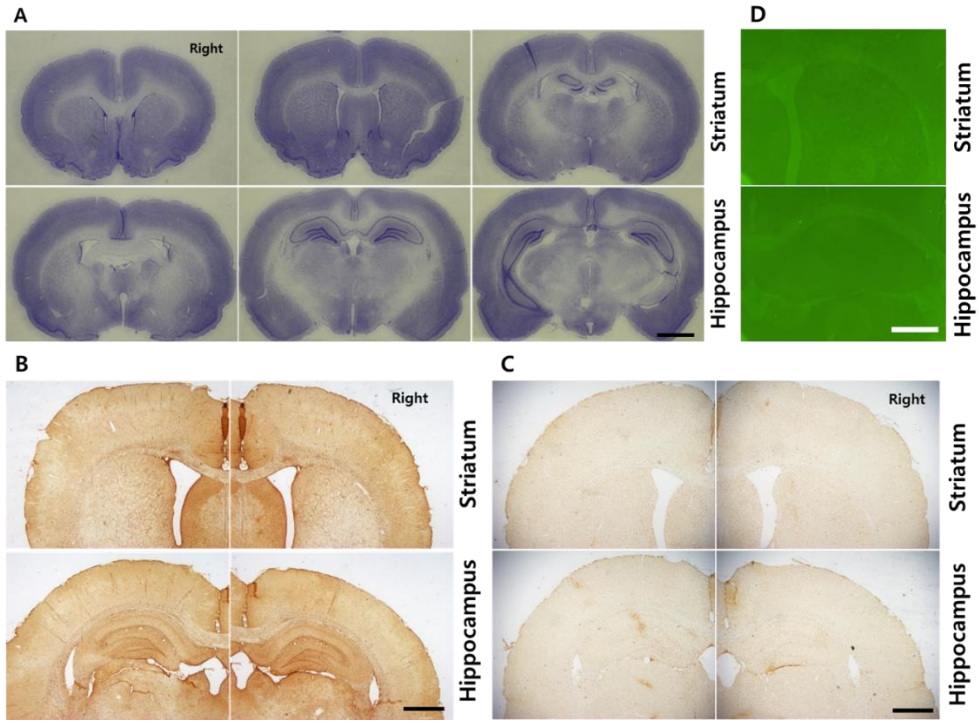

Figure 11. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 600 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

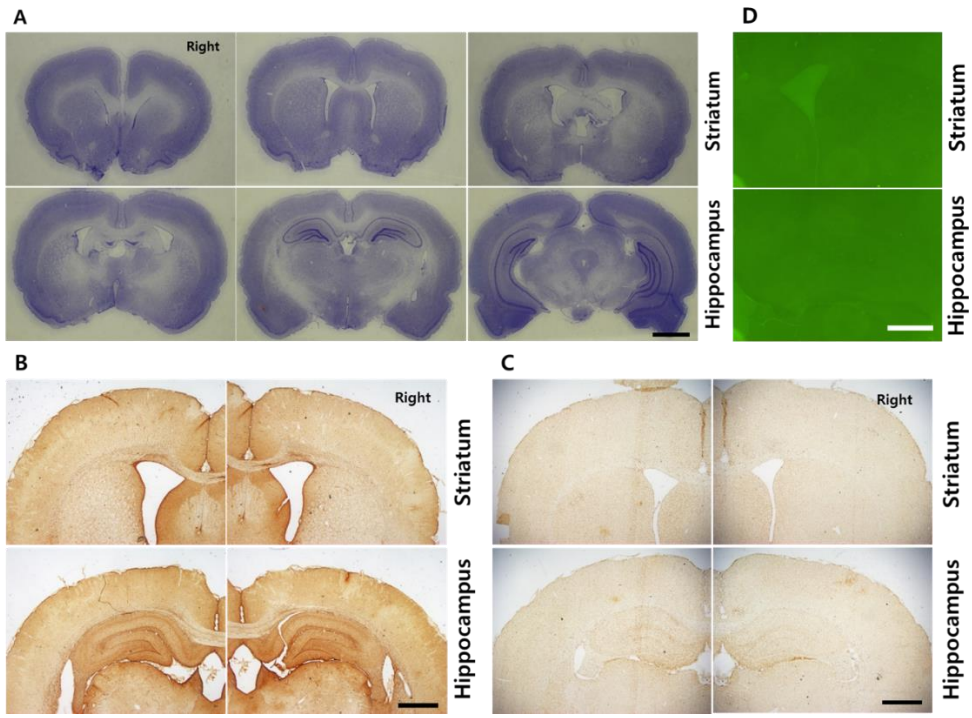

Figure 12. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 600 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

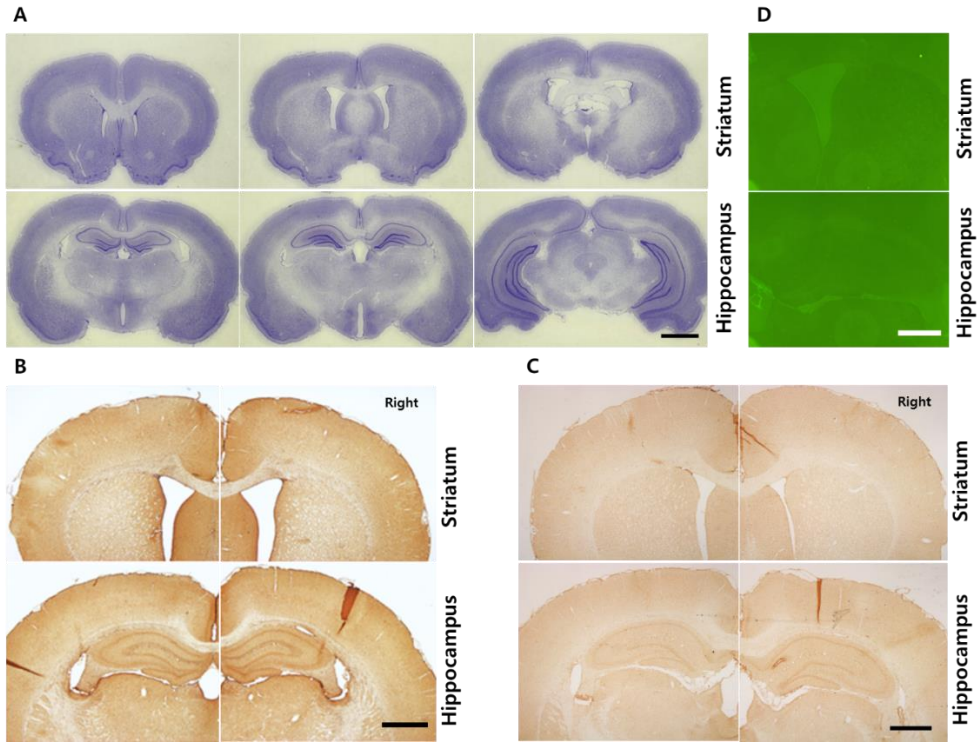

Figure 13. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 2 rat brain on the 1st day after 1200 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

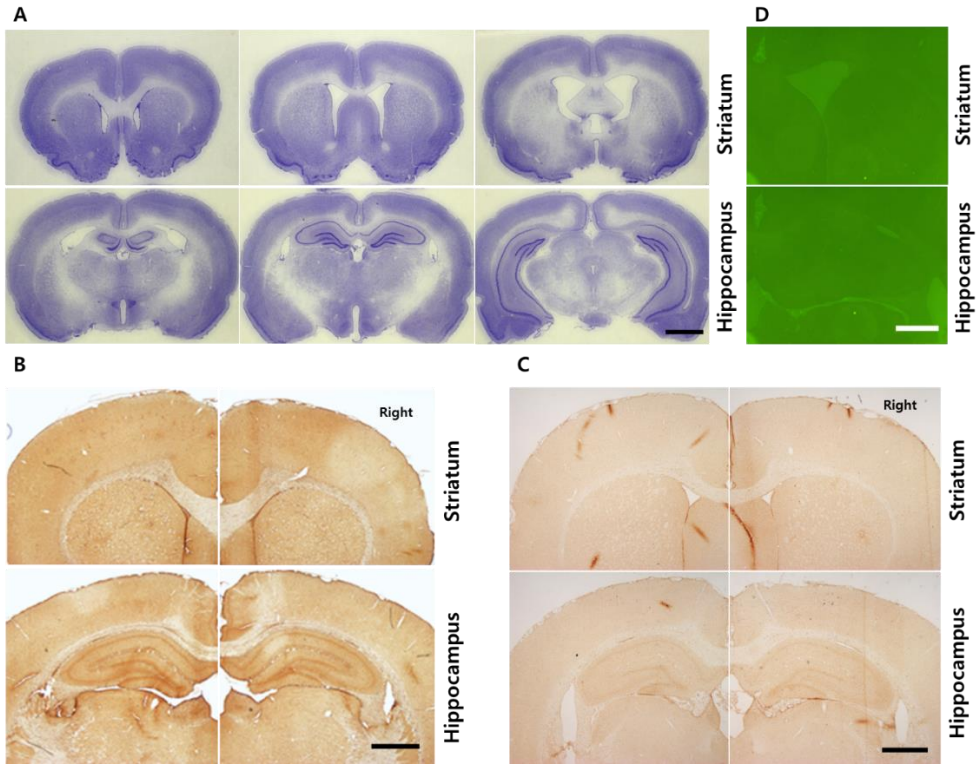

Figure 14. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 1200 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

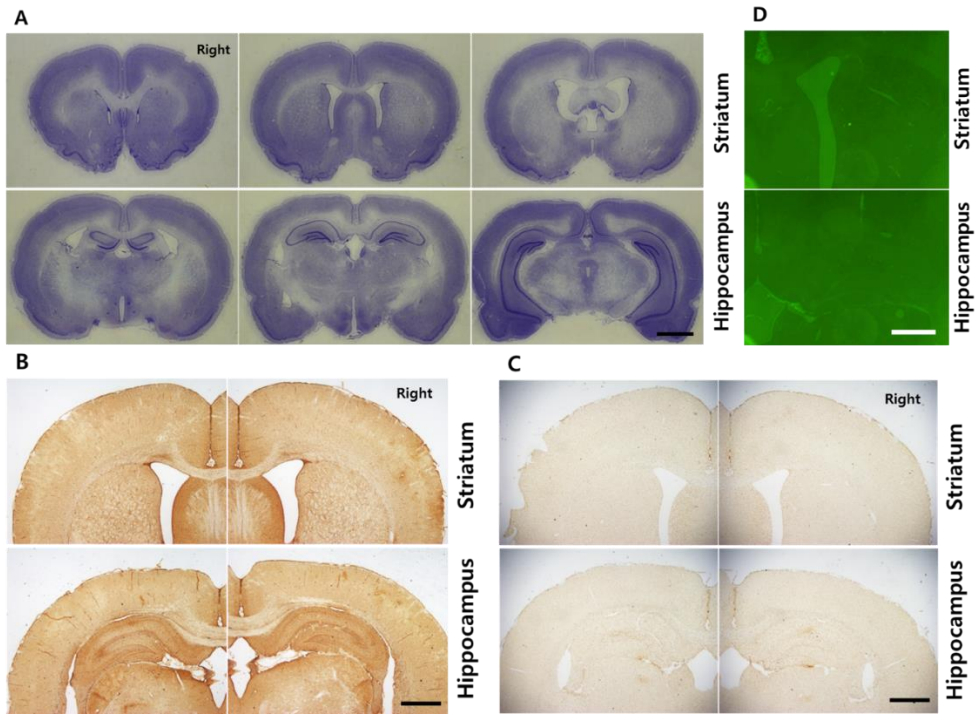

Figure 15. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 1st day after 2400 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

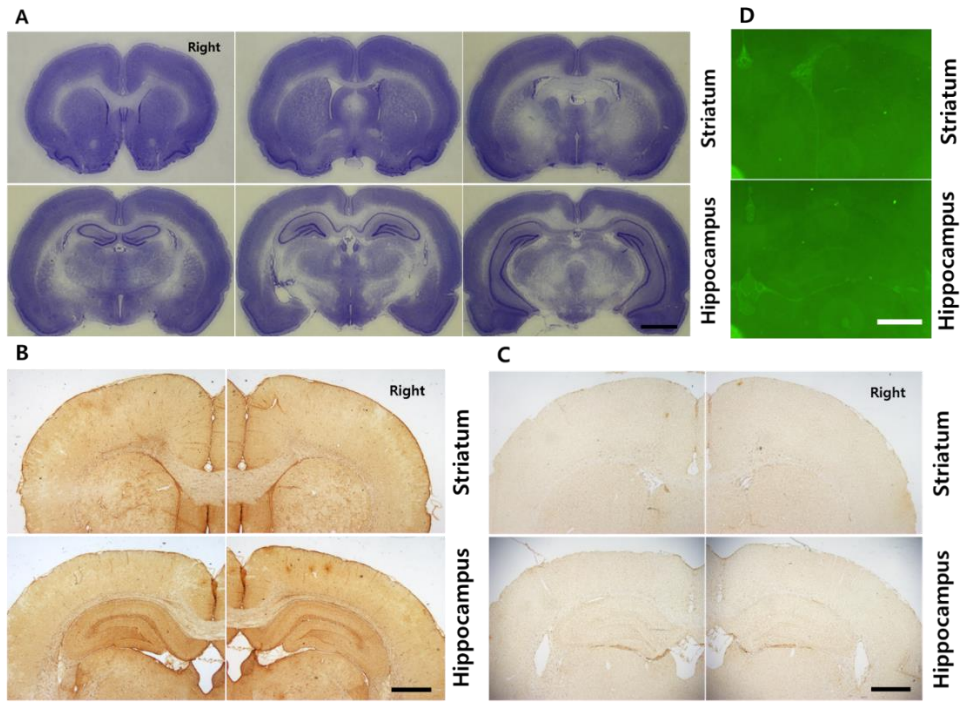

Figure 16. Cresyl violet (A), GFAP (B), Iba-1 (C) and Fluoro-Jade C (D) staining of No. 1 rat brain on the 14th day after 2400 μg human dose of Rapamycin. There are no evidences of brain damage. Scale bar = 2 mm.

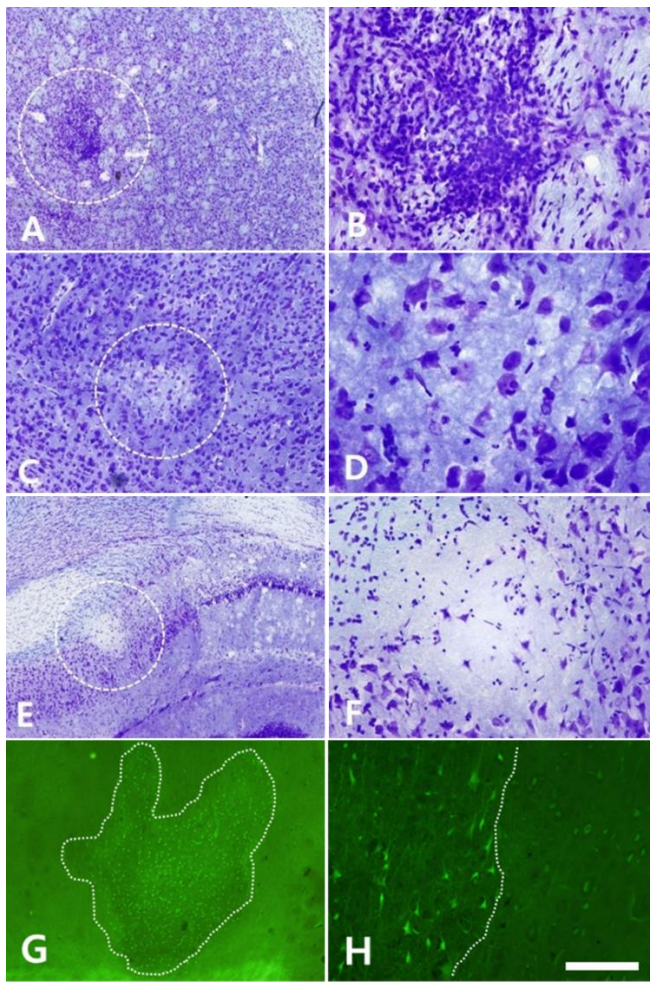

Figure 17. Cresyl violet (A-F) and Fluoro-Jade C (G and H) staining of the No. 4 rat brain on 14th day after 600 μg human dose of Paclitaxel. A and B (magnified photo of dotted circle in A), moderate aggregation of polymorphonuclear inflammatory cells in the striatum. C and D (magnified photo of dotted circle in C), frequent neuronal death in the cortex. E and F (magnified photo of dotted circle in E), frequent neuronal damage in the hippocampus. G and H (partly magnified photo of dotted circle in G), frequent neuronal degeneration in the striatum. Scale bar = 500 μm (E), 200 μm (A and G), 100 μm (B, C, F and H), and 50 μm (D).

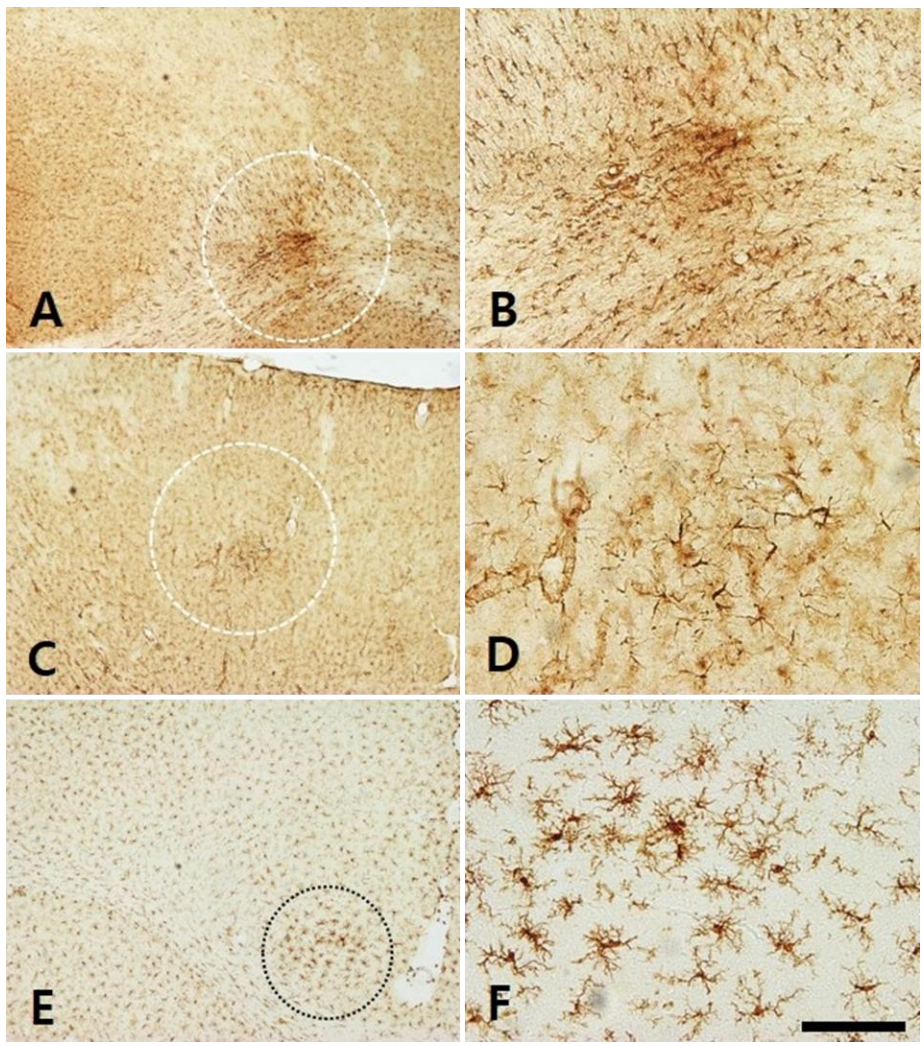

Figure 18. Immunohistochemistry for GFAP (A-D) and Iba-1 (E and F) in the No.4 rat brain on 14th day after 2400 μg human dose of Rapamycin. A and B (magnified photo of dotted circle in A), rarely activated astrocytes in the striatum. C and D (magnified photo of dotted circle in C), rarely activated astrocytes in the cortex. E and F (magnified photo of dotted circle in E), rarely activated microglia in the cortex. Scale bar = 500 μm (A, C and E), 100 μm (B) and 50 μm (D and F).

요약(국문초록)

일시적으로 혈뇌장벽이 열린 래트 모델에서 Paclitaxel과 Rapamycin의 신경독성 연구

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배경: 약물로 조절되지 않는 두개 내 죽상경화성 뇌혈관협착증에 대하여 시술 후 재협착률은 줄이기 위해 약물 방출 스텐트와 풍선이 사용되어 왔다. 그러나, 사용되는 약물의 신경독성 여부에 대한 증거가 아직 부족하다.

목적: 저자들은 일시적으로 혈뇌장벽이 열린 래트 모델에서 약물 방출 스텐트와 풍선에사용되는 약물이 신경독성을 일으키는지 여부를 확인해 보고자 하였다.

방법: 동맥에 직접 약물투여가 가능하도록 우측 경동맥에 카테타를 삽입한 래트 모델을 확립하였다. 만니톨 투여 후 일시적으로 혈뇌장벽이 열

리는 이상적인 시간간격을 탐색하였다. 만니톨을 투여한 뒤 이상적인 시간간격을 두고, 사람에서 600, 1200, 2400 μg에 해당하는 농도의 paclitaxel과 rapamycin을 래트에 투여하였다. 뇌조직은 약물 투여 후 24시간과 14일 후에 얻었는데, 그룹당 5 마리, 총 6 그룹 60 마리를 실험하였다. 모든 래트는 신경학적 및 조직학적 검사를 시행하였다.

결과: 일시적으로 혈뇌장벽이 열리는 최적 시간 간격은 만니톨 투여 후 10분이었다. 경동맥에 만니톨을 투여하고 10분 뒤에 약물을 투여하였을 때, 약물의 종류, 용량 그리고 약물 투여 후 조직검사까지의 시간간격에 상관없이 모든 래트에서 신경독성의 증거는 없었다. 신경학적 이상소견을 보인 래트도 없었다.

결론: 혈뇌장벽이 열린 래트 모델에 정상에서 4배까지 다양한 용량의 paclitaxel과 rapamycin을 경동맥에 직접 투여하였지만 신경독성이 전혀 발생하지 않았다. 이번 동물실험 결과가 향후 인간 대상 치료에 활용되기를 기대한다.

주요어: 두개내 죽상경화성 뇌혈관협착증, 신경독성, Paclitaxel, Rapamycin, 재협착

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